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Sulfide-Oxidizing Symbiosis in Lucinaceans: Implications for Bivalve Evolution

by

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Abstract. Symbiotic sulfide-oxidizing bacteria are present in the bivalve families Solemyidae, Lucinidae, Thyasiridae, Vesicomyidae, and Mytilidae. In *Parvilucina tenuisculpta* the gills have extensive subfilamental tissues composed of storage epithelia and bacteriocytes housing bacteria and many granules of varied composition. Numerous bacteria are present in the gills of *Thyasira flexuosa.* In this species there is less cytological differentiation in the subfilamental tissue. It is proposed that a sulfide-oxidizing symbiosis was responsible for the emergence of the Lucinacea and associated with this symbiosis was a series of paedomorphic events involving gills, siphons, guts, and feeding and ventilatory behavior. The Bivalvia are particularly suitable hosts for sulfide-oxidizing bacteria due to their microphagous habits, their distribution at the interface between aerobic and sulfide-generating anaerobic environments, and their potential ability to control the supply of sulfide and oxygen required by the bacterial symbionts, by partitioning them spatially or temporally. The possible role of sulfide-oxidizing symbiosis in the early evolution of bivalves in relation to the shift from labial palp feeding to ctenidial filtration is discussed.

INTRODUCTION

THE DISCOVERY of symbiotic sulfide-oxidizing bacteria in the vestimentiferan worm *Riftia pachyptila* finally shed light on the perennial question of nutrition in the gutless Pogonophora (CAVANAUGH *et al.,* 1981; SOUTHWARD *et al.,* 1981; SOUTHWARD, 1982). This discovery led also to the identification of symbiotic bacteria in the thermal vent bivalve *Calyptogena magnifica* (CAVANAUGH, 1983), and suggested a solution to questions raised by the gutless condition of the inshore protobranch bivalve species *Solemya reidi* (REID, 1980; REID & BERNARD, 1980). Sulfide-oxidizing enzymes, and enzymes of the Calvin-Benson cycle characteristic of the symbiotic bacteria were identified in these bivalves (FELBECK *et al.,* 1981), and bacteria visualized by transmission electron microscopy were found to inhabit the gills of *Solemya reidi* (FELBECK, 1983) and *Solemya velum* (CAVANAUGH, 1983). Subsequently, similar bacteria have been tentatively identified in all members of the Lucinidae that have been investigated (FELBECK *et al.,* 1981; BERG *et al.,* 1983; BERG & ALATALO, 1984; FISHER & HAND, 1984; SCHWEIMANNS & FELBECK, 1985; DANDO *et al.,* 1985). It is inferred that the bivalve controls the availability of oxygen and sulfide for the bacteria in these associations, and that the bivalve benefits from the detoxification of sulfide and the translocated carbohydrate and amino acid products of the bacterial metabolism.

In this report *Parvilucina tenuisculpta* (Carpenter, 1864), a bivalve belonging to the Lucinidae, which has already been shown to possess symbiotic bacterial enzymes (FELBECK *et al.,* 1981) is taken as a model for the study of the impact of the symbiosis on bivalve functional morphology. Two species of the related family Thyasiridae are also studied, and drawing upon the extensive survey of the Lucinacea conducted by ALLEN (1958) we re-interpret the evolution of this group in the context of the symbiosis with sulfide-oxidizing bacteria. Furthermore we report on a TEM survey of other bivalves inhabiting sulfide substrates and consider some of the general implications of these symbioses for bivalve evolution.

MATERIALS AND METHODS

Specimens of *Parvilucina tenuisculpta* (Carpenter, 1864) were collected with a Van Veen grab from fine silt with a strong odor of hydrogen sulfide at a depth of 40 m in Pipestem Inlet, Barkley Sound, W. Vancouver Island, British Columbia. For a discussion of the taxonomic status of the genus *Parvilucina* refer to BRITTON (1972). Other numerous mollusks from this environment included *Macoma carlottensis* (Whiteaves, 1880) and *Dentalium rectius*

(Carpenter, 1864). Specimens of the two bivalve species were transported to the University of Victoria and allowed to burrow in mud from the biotope contained in glass vessels submerged in circulated seawater. *Axinopsida serricata* (Carpenter, 1864) and *Thyasira flexuosa* (Montagu, 1803) were obtained at a depth of 30 m off the Crofton pulp mill, S.E. Vancouver Island. A preserved specimen of the E. Pacific hydrothermal vent mytilid was borrowed from V. Tunnicliffe for a general anatomical scrutiny. Specimens of *Compsomyax subdiaphana* (Carpenter, 1864), *Acila castrensis* (Hinds, 1843), *Yoldia scissurata* (Dall, 1897), *Macoma lipara* (Dall, 1916), *M. brota* (Dall, 1916), *M. calcarea* (Gmelin, 1791), and *M. elimata* (Dunnill & Coan, 1968) were obtained by dredging from silt substrates at 30 to 60 m off Moresby Island, S.W. British Columbia. *Macoma nasuta* (Conrad, 1837), *M. secta* (Conrad, 1837), and *M. inquinata* (Deshayes, 1855) were dug intertidally at Cordova Bay, S.E. Vancouver Island.

The morphology of *Parvilucina tenuisculpta* was examined by dissection and by serial sectioning. Ciliary currents in the mantle cavity were traced with suspensions of alumina (about 30 μm), 280-mesh carborundum (35–75 μm), and Sephadex G10 beads (60–120 μm).

Freshly collected specimens were removed from the shell and fixed in cold, neutral paraformaldehyde fixative (HUMASON, 1979) for 3 h at 8°C, then infiltrated and embedded in JB4 plastic at 8°C. The plastic blocks were stored dry at 4°C until sectioned. Sections 2 μm thick were cut from these blocks and subjected to incubating and staining media for the visualization of non-specific esterases and acid and alkaline phosphatases (REID, 1966). Some of the blocks were used for serial sectioning, with sections being stained with Mayer's haematoxylin and xylidene Ponceau. Selected slides were stained with alcian blue to reveal acid mucopolysaccharides, or with periodic-acid-Schiff (PAS) for general staining of polysaccharides. Diastase-treated sections provided a control for the presence of glycogen in PAS-stained sections.

Freshly dissected gills of *Parvilucina* and gills and labial palps of the other bivalves mentioned above were prepared for transmission electron microscopy (TEM) according to the following protocol based on EISENMAN & ALFERT (1982): 10 min prefixation in a medium consisting of 1 part postfix to 20 parts mainfix, followed by 12 h fixation in mainfix consisting of 4% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2), and 0.35 M sucrose, followed by 1 h postfixation in postfix consisting of 1% osmium tetroxide, in 0.3 M sodium chloride, 0.2 M sodium cacodylate buffer, and 0.35 M sucrose. Dehydrated samples were embedded in epon (EMbed 812). Sections were examined in a Philips EM 300 microscope.

For scanning electron microscopic examinations (SEM) freshly dissected gills of *Parvilucina tenuisculpta* were fixed for 2 h in 3.5% glutaraldehyde in 0.1 M sodium phosphate buffer and 3.5% sucrose (pH 7.2), followed by postfixation for 1 h in 1% osmium tetroxide in 0.1 M phosphate buffer and 3.5% sucrose at 4°C. Dehydrated specimens were critical point dried, gold coated, and examined in a JEOL JSM-35 scanning electron microscope.

For examination of granular inclusions by energy dispersive X-ray microanalysis (EDX) dissected gills were homogenized in a Polytron sonic/mechanical homogenizer and centrifuged at 10,000 rpm for 10 min and then for two 10-min periods at 5000 rpm. After each centrifugation the supernatant was discarded and pellets were resuspended in deionized water. The final resuspension was filtered through 0.2-μm polycarbonate nucleopore filters. The filters containing the granules were dried, carbon coated, and examined in the SEM mode of a JEOL JEM 1200 EX microscope, and X-ray analysis was conducted by means of a Tracor X-ray detector linked to a Tracor-Northern computer. Semiquantitative analysis of the proportions of metals in the granules were made following methods based on Kramer's model, outlined by GOLDSTEIN *et al.* (1981), and programmed by Tracor-Northern TN-5550-10 Quantitative Software for Scanning Electron Microscopes and the TN-5500 X-ray Analyzer, 1984.

For the detection of hemoglobin, aqueous extracts of whole body homogenates were centrifuged at 12,000 rpm and 4°C for 2 h. The supernatants were scanned spectrophotometrically to detect absorption peaks between 400 and 700 nm. The effect of deoxygenation by 1% sodium dithionate on the absorption profile was also observed.

RESULTS

Functional Morphology of *Parvilucina tenuisculpta*

The pallial morphology of *Parvilucina tenuisculpta* has many features in common with the other members of the Lucinidae. There is no inhalant siphon; the exhalant siphon is short and can be inverted so that its opening comes into close contact with the suprabranchial chamber; the foot, a long organ with a pointed expanded tip, can be extended many times the length of the shell; and the anterior adductor is elongated ventrally (Figure 1). The lateral extensions of the visceral mass, containing part of the reproductive organs, form distinct pouches, and the ducts and tubules of the digestive gland are placed superficially over the pouches (Figure 2). The much thickened ctenidia are the most striking feature of the pallial morphology. These consist of single demibranchs. The individual gill filaments, which have longitudinal streaks of brown and yellow-brown pigment, are held close together by multiple tissue bridges. A distinct, but shallow food groove is present at the ventral margin of each demibranch (Figure 3). For most of their length the inner, ascending lamellae are connected by a thin septum to the visceral mass. Posteriorly the dorsal margins of the gills are fused to the mantle edge, as shown in Figures 1 and 2. The descending gill filaments are fused to the opposite ascending filaments for most of their length through extension of the granular, pigmented subfilamentar tissue, effectively combining the filaments into narrow blades arranged like the teeth of a comb. Alumina particles, carborundum particles, and

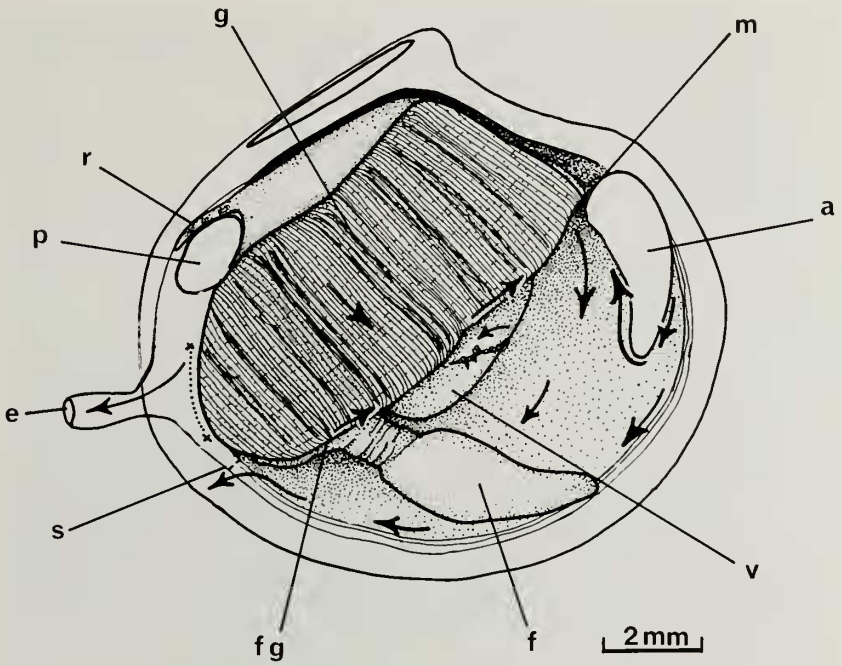

Figure 1

Right lateral view of *Parvilucina tenuisculpta,* mantle removed. Dotted line under the posterior adductor muscle indicates the region where the dorsal edges of the outer gill lamellae are fused to the mantle edge. a = anterior adductor muscle; e = exhalant siphon; f = foot; fg = food groove; g = gill; m = position of mouth; p = posterior adductor muscle; r = rectum; s = point of separation of fused mantle edges, where pseudofeces are ejected; v = visceral mass.

Sephadex particles smaller than 75 μm are swept over the surface of the gill and carried in mucus-bound strings to the mouth. The labial palps are reduced to a narrow pair of dorsal and ventral lips, together with two small papillae arranged at each end of the lips adjacent to the food groove (Figure 2). The gills communicate with the mouth by isthmuses whose cilia carry food particles from the ctenidial food grooves to the mouth. The ciliation of the lips and palp papillae appear to have a cleansing function only. Serial sections indicate a slight folding of the mantle epithelia in the region of the mouth and anterior adductor muscle but there is no distinct pallial gill, such as ALLEN (1958) describes for other lucinids. A surface ciliation of the anterior adductor muscle carries particles in the direction of the mouth but it is not clear if there is any direct contact between particles collected in this manner and the mouth. The mantle edges are separate for the anterior two-thirds of their length. Posteriorly they are fused and form an elastic ribbon that can relax to about four times its contracted width. Its musculature is complex, consisting of transverse, radial, longitudinal, and oblique fibers. As noted above, the postero-dorsal descending gill filaments are connected to this muscular organ. Although we did not observe its manner of operation, its contraction must have a bellows-like effect on the gills, thereby agitating or flushing the mantle and suprabranchial water. Rejectory currents on the inner mantle surfaces carry particles of pseudofeces to the point where the mantle edges fuse (Figure 1).

The stomach of *Parvilucina tenuisculpta* is relatively small, and its internal morphology is simple, similar to that of the lucinid *Lucinoma borealis* (PURCHON, 1958). The major typhlosole is a thin ribbon of tissue, adjacent to the intestinal groove, which continues ventrally into the mid-gut, open to the style sac. Particles retrieved from the stomach included silt, small diatoms, and organic detritus in the size range of 5–40 μm. There appear to be four duct openings giving rise to four diverticular ducts, each of which gives rise to up to eight digestive tubules. The ciliated epithelia of the ducts give a positive reaction for alkaline phosphatase. The tubule cells are positive for acid phosphatase and non-specific esterase. A relatively short mid-gut continues into a short hind-gut, ending in a rectum that carries fecal pellets dorsal to the posterior adductor to the exhalant siphon.

Whole-body aqueous extracts submitted to scanning spectrophotometry show absorption peaks at 500, 538, 569, and 608 nm. When the extracts are deoxygenated with sodium dithionate the absorption peaks shift to 532 and 565 nm. These absorption peaks and their deoxygenation

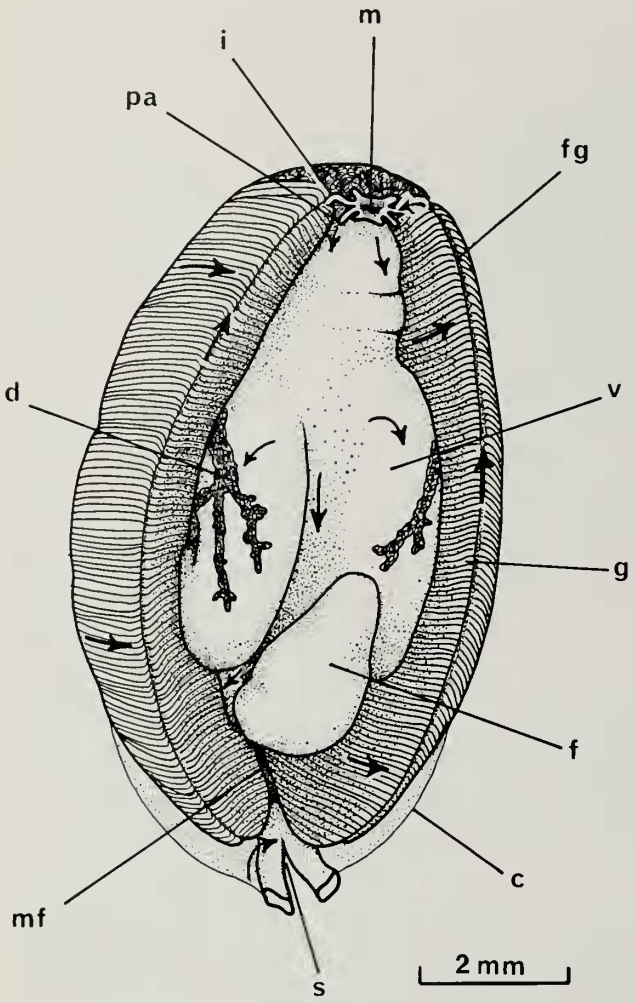

Figure 2

Anteroventral view of *Parvilucina tenuisculpta,* removed from valves, mantle removed. c = cut edge of mantle; d = digestive diverticula; f = foot; fg = food groove; g = gill; i = isthmus of ciliated tissue connecting ctenidial food groove to mouth; m = mouth; mf = region of fusion of dorsal margins of gills to mantle edge; pa = vestigial papilla of labial palp; s = point of separation of fused mantle edges, where pseudofeces are ejected; v = visceral mass.

shifts are similar to those found for the hemoglobin of the lucinid *Phacoides pectinatus* by READ (1962) and in *Solemya reidi* (MCMAHON & REID, 1984; DOELLER & COLACINO, 1985).

Fine Structure and Inclusions of the Gills of *Parvilucina tenuisculpta*

Due to fusion of the subfilamental (or abfrontal filamental) tissues the demibranchs of *Parvilucina tenuisculpta* form a series of narrow lamellae that are formed of three cell types (Figure 4). The cells of the frontal region are typical of the normal bivalve filament, being ciliated and containing numerous mitochondria (Figures 4A, 5A). Adjacent to these are large cells whose inclusions consist of a granular mucopolysaccharide that stains weakly with alcian blue and strongly with PAS. Numerous granules of glycogen are also present. This tissue will be called *storage epithelium* (Figures 4, 5, 6). The remainder of the subfilamental septum consists of bacteriocytes that contain a variety of large granules, which vary in appearance under the light microscope, and are colored various shades of yellow, orange, and brown. Alternating with the bacteriocytes are small single *intercalary cells* with extensive microvilli on their distal surfaces. This is the term used by DANDO *et al.* (1985), who report a similar arrangement in *Myrtea spinifera* (Montagu). Alternating intercalary cells are also found by us in *Solemya reidi,* but are sometimes obscured by poor fixation. As Dando *et al.* note, the microvillar surface of the intercalary cells partially extends over the bacteriocytes. These cells may divide to produce new bacteriocytes, and may also have a transport role linked to bacterial metabolism. The bacteria in the bacteriocytes are contained in distinct vacuoles, as in *Myrtea* (Figures 4D, 5A, B, C). FISHER & HAND (1984) report a similar bacterial type in vacuoles in the gill bacteriocytes of *Lucina floridana.* Some bacteria are found extracellularly in the spaces between the gill lamellae, together with free granules (Figure 5C). These may have come from the degradation of senescent bacteriocytes, or may have been actively exocytosed. Amoebocytes found between the bacteriocytes and in the ctenidial blood sinuses contain a few bacteria and granular inclusions, some of which have a distinctly membranous appearance (Figure 6A).

There are various types of granular particles in the bacteriocytes (Figures 6B, C). Granules with low sulfur, silicon, phosphorus and calcium, and relatively high chromium, iron and nickel are the most common type in *Parvilucina* (Figure 7A). The presence of the calcium and phosphorus suggest that these may be nephrolith-like bioaccumulation granules that have sequestered iron, nickel, and chromium from the water column or from the blood (TIFFANY, 1982; REID & BRAND, 1985). EDX microanalysis demonstrates that sulfur is the dominant elemental inclusion of the bacteria themselves (Figure 7B). In some granules, sulfur predominates, but significant levels of chromium, iron, and nickel are also present (Figure 7C). In contrast, the type of granule shown in Figure 7D has the elemental make-up of plagioclase feldspar, a common constituent of sand and silt in the environment. A few fine particles of this type may have been accidentally endocytosed by the gill epithelia, or may simply have adhered to the surface of the gill prior to preparation for EDX analysis. GAILL *et al.* (1984) note that sulfur in the hydrothermal vent polychaete *Alvinella pompejana* is associated with high levels of zinc and arsenic, and conclude that these metals may have a biological role in bacterial metabolism. They also report the presence of arsenic in *Calyptogena magnifica* from the same community. Zinc

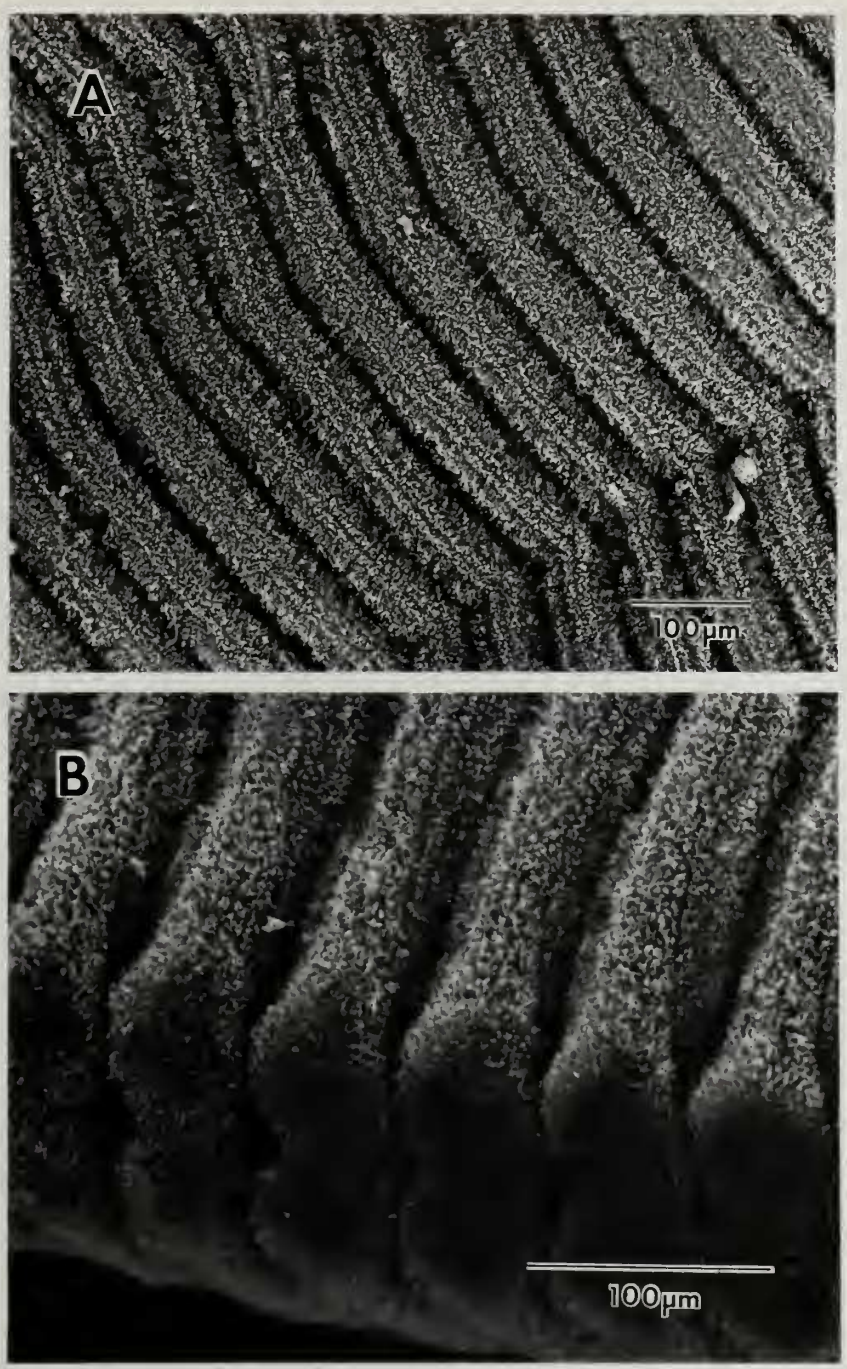

Figure 3

Scanning electron micrographs of gills of *Parvilucina tenuisculpta*. A. Lateral view of filaments. B. Ventral margin showing weak food groove.

could, they allow, be absorbed from the water column without any special biological power of concentration. Arsenic is not detected in *Parvilucina tenuisculpta,* and the zinc that is present may come directly from the water column. Most of the sulfur present in *Parvilucina* granules is probably the end product of bacterial metabolism. However, sulfur is also present in the numerous cysteine residues of the metal-binding protein metallothionein. The

precise role of these proteins in metal deposition in bivalve granules is not yet defined. GEORGE (1983a, b) notes that, although metallothionein is involved in cadmium detoxification in *Mytilus,* the sulfur present in the accumulation granules cannot be traced to cysteine derived from the protein. Table 1 provides the data from a semi-quantitative analysis of the inorganic heavy elements from these particles together with a rough percentage of the common granule types. Owing to the significant environmental levels of iron in the sediment and in the water column it should not be assumed that the iron content of these granules is correlated with respiratory pigments or cytochromes.

The Gills of *Thyasira flexuosa* and *Axinopsida serricata*

Both of these species of the lucinacean family Thyasiridae have extensive subfilamental tissues. In *Axinopsida* these tissues are similar to the storage epithelium of *Parvilucina* (Figure 8A). There are occasional inclusions that might be bacteria, but these are scarce, and there are no specialized bacteriocytes. In *Thyasira* it appears as if the storage epithelia double as bacteriocytes. Numerous bacteria are contained in large vacuoles, or "bacteriosomes," at the distal surfaces of these cells (Figure 8B). This creates the impression that formerly epifloral gill symbionts were phagocytosed and the symbionts now conduct their affairs from within a phagosome. However, we find no extant epiflora remaining on the gill surfaces. Some of the bacteria appear to be further ingested into the storage cell proper (Figure 8C). This arrangement suggests that sulfide is taken in through the microvillar distal surfaces of the subfilamental epithelium, rather than from the blood.

TEM Survey of the Gills of Eleven Bivalve Species

Only one of these species showed evidence of gill bacteria. An extensive survey of *Macoma* was conducted because *M. carlottensis* is often found along with *Solemya reidi, Parvilucina tenuisculpta,* and *Thyasira flexuosa.* JONES & THOMPSON (1984) list the common faunal associates of *P. tenuisculpta.* A number of other *Macoma* spp., in particular *M. inquinata, M. lipara, M. brota,* and *M. calcarea,* inhabit sulfide silts and we had the unrequited hope that this study would solve a problem of niche partition pertaining to *Macoma* (REID & REID, 1969). *Compsomyax subdiaphana* is another common bivalve of sulfide silts in the northeastern Pacific. *Acila castrensis* was chosen because of its similar habitat and its central role in discussions of the evolution of habit in the bivalves. However, with all of these species we drew blanks. Only in *Yoldia scissurata* did we find some ctenidial cells containing bacterium-like inclusions somewhat similar to those of *Axinopsida serricata.* These inclusions were found in only a few cells, and not in the kinds of arrays of bacteriocytes found in *Parvilucina, Thyasira,* and *Solemya.* Granules in the gills of *Yoldia* did not have high sulfur levels.

DISCUSSION

The Functional Morphology of *Parvilucina tenuisculpta*

The features of the functional morphology of *Parvilucina tenuisculpta* that may be correlated with the bacterial

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Figure 4 (page 9)

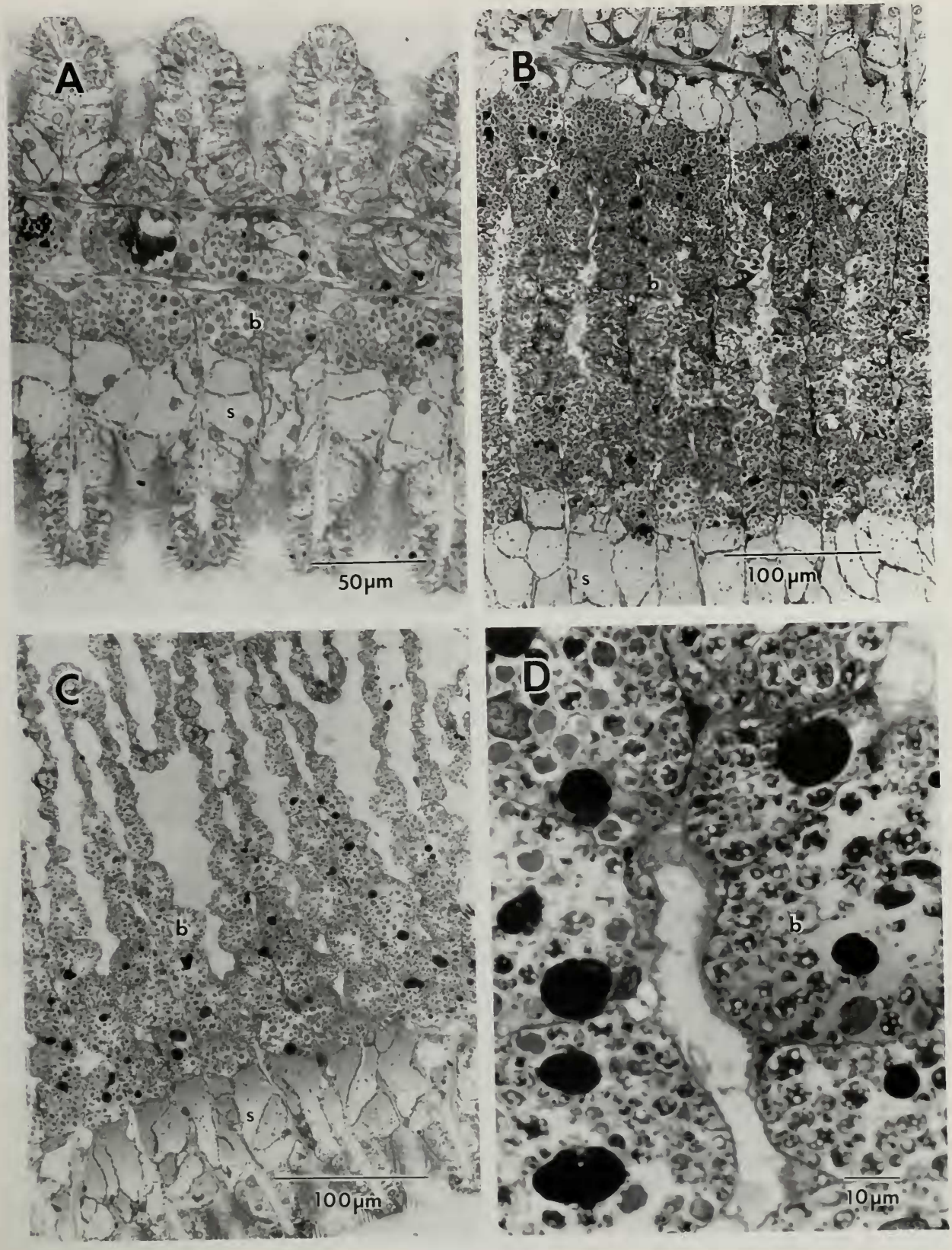
Light micrographs of thick epon sections of gill filaments of *Parvilucina tenuisculpta.* A. Section near ventral margin showing the ciliated frontal portions of filaments, the bacteriocytes (b) with large granules, and between these the storage epithelia (s). B. Section in central region of gill showing transverse fusion of the ascending and descending filaments. The subfilamental (abfrontal) tissue consists of bacteriocytes and intercalary cells. C. Section near dorsal region of gill. Gill filaments are no longer fused transversely. D. Higher magnification of bacteriocytes showing large mineral granules and bacteria.

Figure 5 (page 10)

Transmission electron micrographs of gill filaments of *Parvilucina tenuisculpta.* A. Frontal epithelia with cilia and numerous mitochondria. B. Bacteriocyte with bacteria (ba). C. Bacteriocytes; note the microvillar borders and free bacterium in suprabranchial space between the cells (indicated with arrow). D. Amoebocyte between storage cell (upper left) and bacteriocyte (lower right); amoebocyte contains bacteria (ba) and has mitochondria.

Figure 6 (page 11)

Granular deposits in gill filaments of *Parvilucina tenuisculpta.* A. Amoebocyte in filamental blood sinus contains compound lamellar inclusion. B. Dense granule from bacteriocyte, may be older form of C. C. Compound lamellar granule of bacteriocyte. Note that these granules do not have the same ultrastructure as the calcium phosphate nephroliths mentioned in the text.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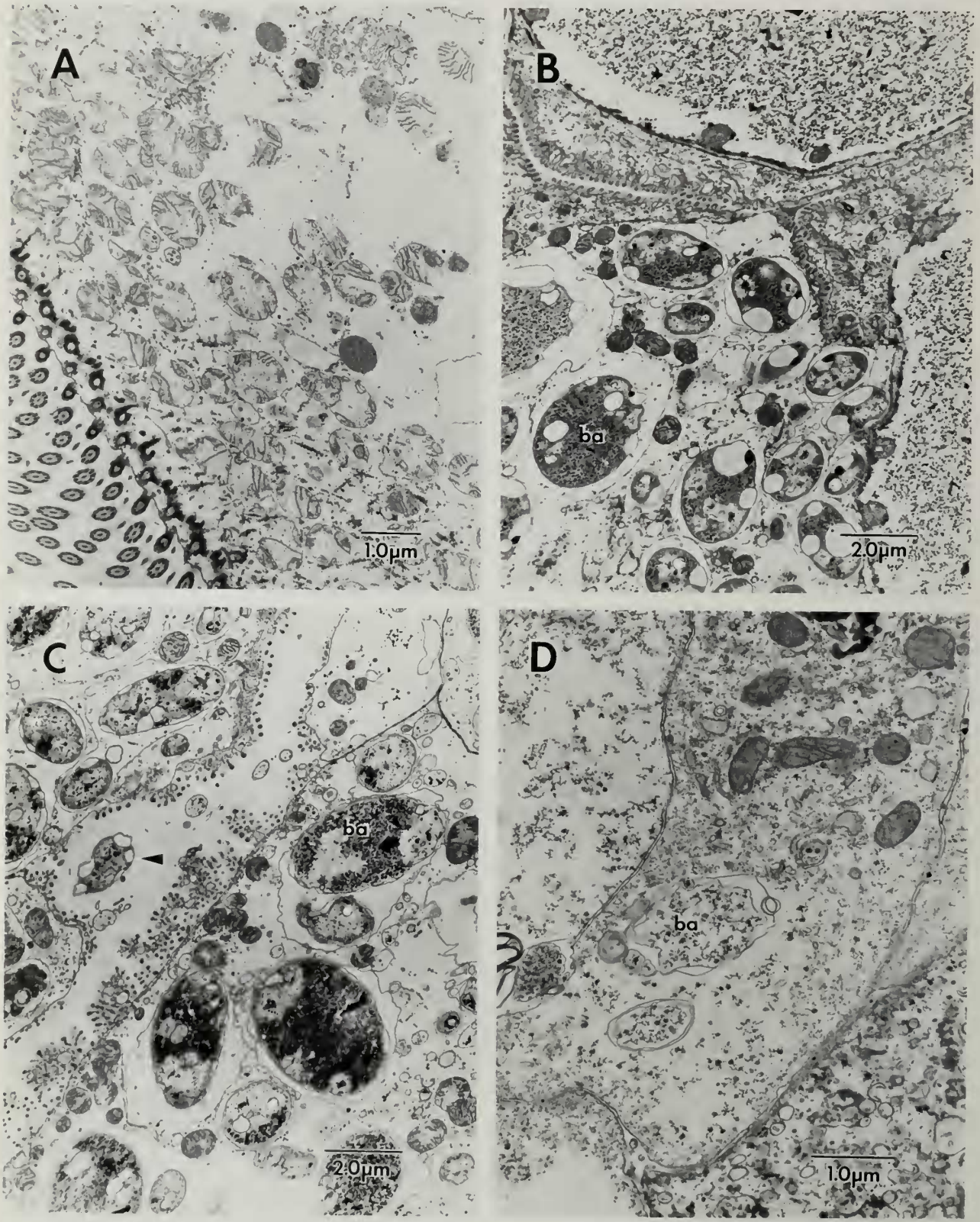
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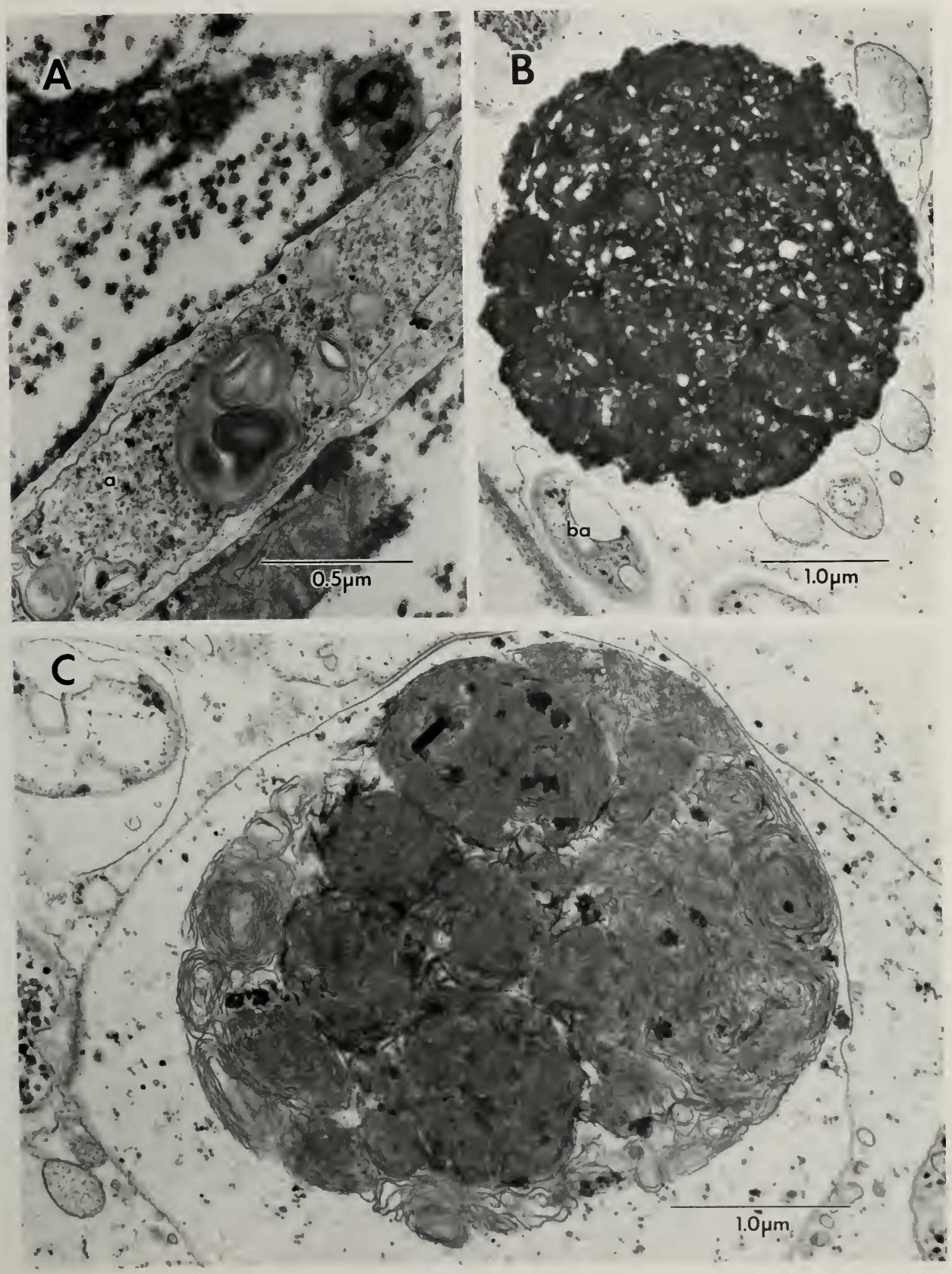
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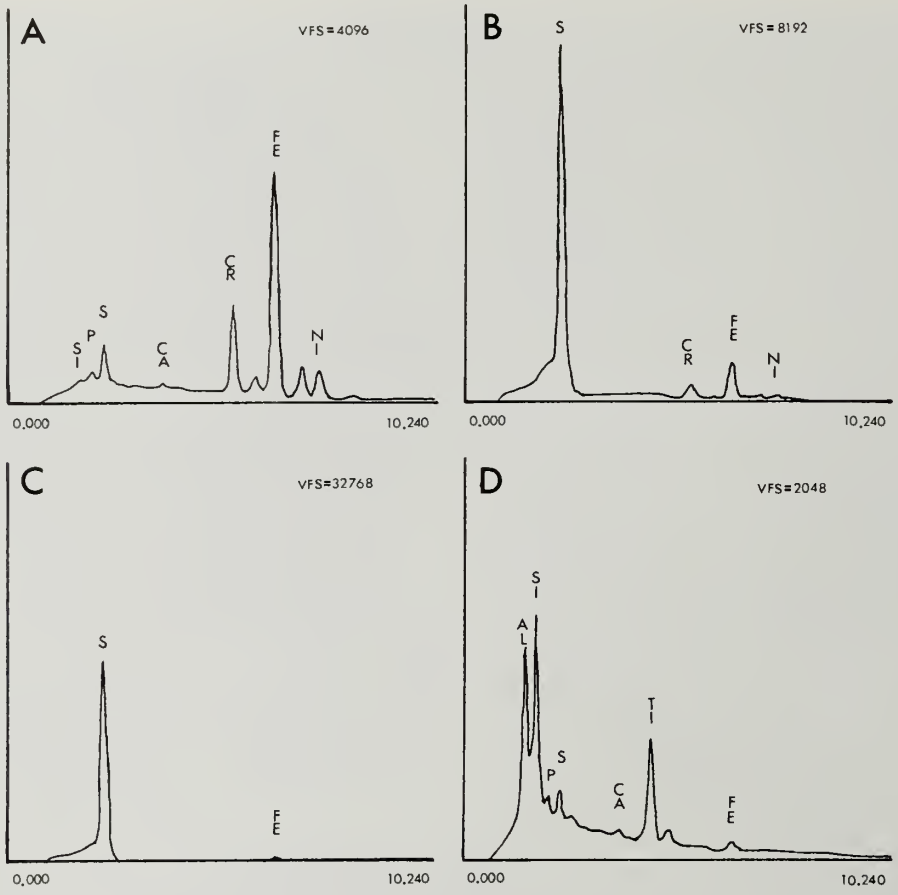


Figure 7

EDX assays of mineral granules from the gills of *Parvilucina tenuisculpta*. A. The most common type of granule characterized by high iron, chromium, and nickel. B. Granule with combined features of iron-chromium-nickel granule and bacterial sulfur granule. C. Bacterium. D. Plagioclase feldspar granule (artifact from environment). Note that the height of the peaks is not proportionate to element concentration. For semi-quantitative analysis refer to Table 1.

symbiosis include the expansion of the subfilamental tissue of the gills, the insertion and fusion of the posterodorsal margins of the gills with the muscular posterior mantle edge, the reduction of the labial palps, the hypotrophy of the gut, the capacity to construct a ventilation burrow, and the absence of an inhalant siphon. The absence of the outer demibranch is also significant, though not exclusively correlated with symbiosis. The probable presence of hemoglobin and the deposition of sulfur granules in the gills are additional correlations. Among these features only the posterior fusion of the gills with the mantle edge has not been reported for the Lucinidae. Because the gills are not themselves muscular the contraction of the mantle edge may give the gills a bellows-like action to bring solutes such as sulfide from the immediate external environment. Conceivably this arrangement could cause a tidal flushing of the suprabranchial chamber via the exhalant siphon, thus bringing sulfide into immediate contact with bacteriocytes, while limiting exposure of the ciliated frontal cells. In all probability the lack of other instances of this arrangement in the Lucinacea is not due to its uniqueness in *Parvilucina tenuisculpta,* but rather that it has been overlooked elsewhere. From this point the discussion will generalize about the Lucinidae.

Uptake of Sulfide and Oxygen

The Lucinidae often show one character that is not developed in *Parvilucina tenuisculpta,* the mantle gills. In many lucinids these are composed of vascularized diagonal folds, which Allen (1958) interprets as supplementary oxygen-absorbing organs. This could be a major point of oxygen uptake or, in some cases, sulfide absorption. Hemoglobin was first discovered in the Lucinidae by Read (1962) and is probably present in *Lucina floridana* (Fisher & Hand, 1984) as well as in *Parvilucina tenuisculpta.* The bivalve requires oxygen and the bacterial symbiont re-

Table 1

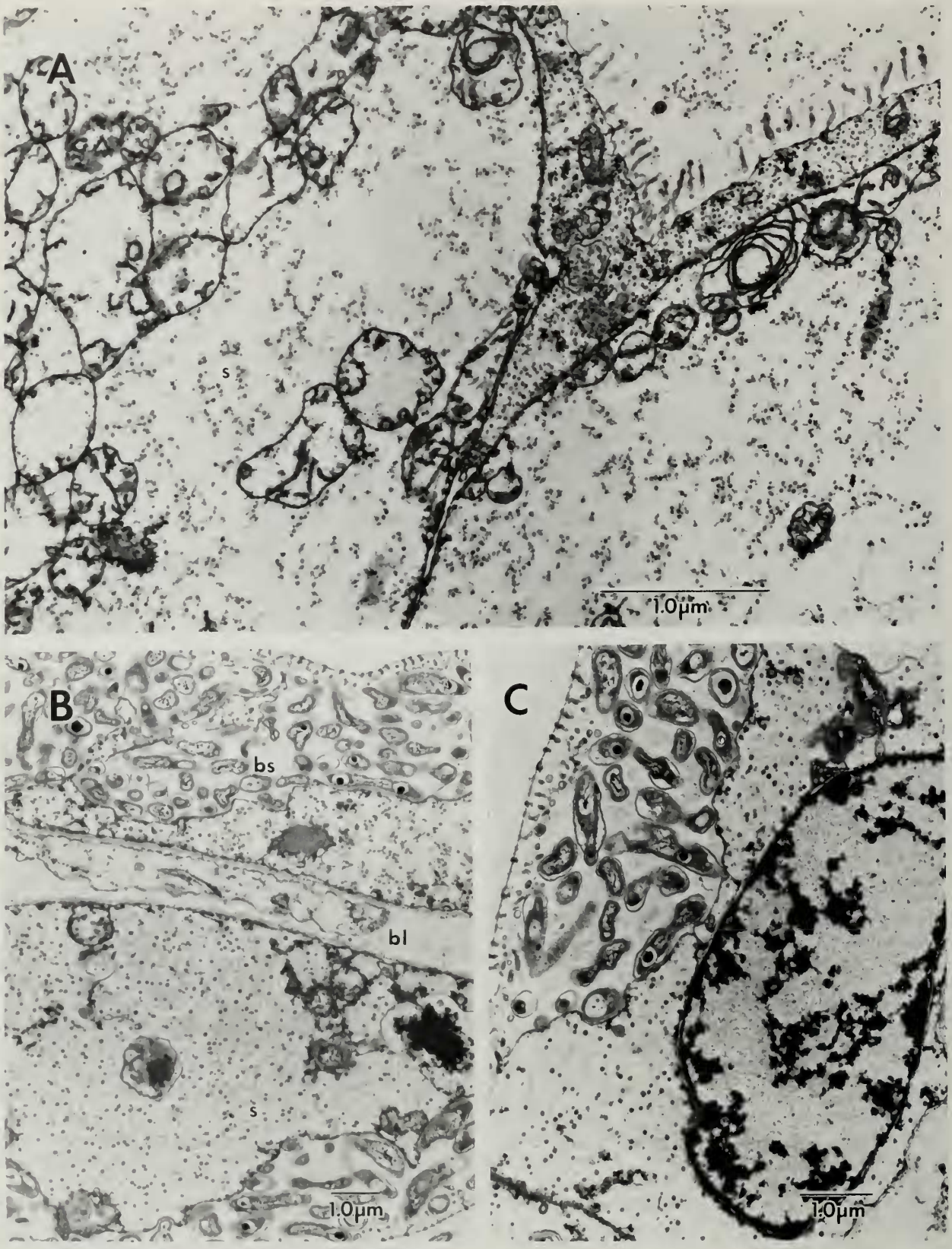
Percent weight of heavy elements of ctenidial granules of *Parvilucina*.

Al	Si	P	S	Ca	Ti	Cr	Fe	Ni	% of 25 granules examined
0.00 ±0.00	0.83 ±0.41	2.29 ±1.61	2.21 ±0.96	0.00 ±0.00	0.00 ±0.00	15.01 ±1.82	64.82 ±1.52	14.91 ±1.41	28
0.00 ±0.00	1.46 ±1.02	11.32 ±4.16	60.75 ±6.60	1.34 ±2.17	0.00 ±0.00	1.33 ±1.82	19.85 ±5.86	4.27 ±0.56	32
0.00 ±0.00	1.68 ±0.92	5.47 ±1.56	86.00 ±8.24	5.05 ±9.97	0.00 ±0.00	0.46 ±0.65	2.33 ±2.67	0.69 ±0.88	24
21.67 ±0.47	32.64 ±0.88	8.85 ±1.02	9.69 ±0.79	0.00 ±0.00	23.40 ±1.03	0.00 ±0.00	3.38 ±0.52	0.37 ±0.08	8
0.00 ±0.00	0.34 ±0.47	0.94 ±0.13	3.30 ±0.59	92.39 ±3.59	0.00 ±0.00	0.32 ±0.45	2.67 ±3.38	0.30 ±0.12	8

quires both oxygen and sulfide. Because the two molecules rapidly inter-react, a spatial or temporal separation is required. Spatial separation could be achieved by separate routes of ingress to the bacteriocytes, one route being direct diffusion from the mantle cavity and the other from the blood and hemoglobin. As already suggested, sulfide could be brought directly to the bacteriocytes in the suprabranchial chamber via the exhalant siphon. The juxtaposition of bacteriosomes and storage epithelia in *Thyasira* suggest a similar route for sulfide absorption rather than from the blood. Temporal partition of the oxygen and sulfide supply could be achieved, as proposed for *Solemya reidi,* by changes in ventilatory behavior (McMahon & Reid, 1984). *Solemya reidi* can cease ventilation but still remain open for the absorption of sulfide accumulated in the burrow. After the gill bacteriocytes have been loaded with sulfide, the restoration of ventilation would supply the required oxygen. Doeller (1984) pointed out that *Solemya velum* could achieve the same ends by moving in its burrow between the aerobic and anaerobic zones. Doeller & Colacino (1985) have indicated a mechanism in *Solemya velum* and *S. reidi* that integrates the spatial and temporal alternatives for the separation of oxygen and sulfide. In these species hemoglobin, which has a moderate to high oxygen affinity, binds sulfide reversibly, and thus can act as a store providing sulfide while the organism is in an aerobic environment, and providing oxygen when in an anaerobic environment. McMahon (personal communication) has found that in a respiration chamber where sulfide is not available and environmental oxygen has all been absorbed, *S. reidi* releases oxygen back into the environment. Presumably, under natural conditions in an anoxic environment sulfide would be present and the oxygen from the hemoglobin would be used for bacterial metabolism. The Lucinidae do not have the option of ascending and descending in the burrow between the aerobic and anaerobic zones. However, one common feature of the Solemyidae and Lucinacea is the absence of a tubular inhalant siphon that would physically impede sulfide absorption, both when extended and contracted. One interesting ecological twist is the photosynthetic oxygen acquired by *Lucina floridana* from the roots of eelgrass (Fisher & Hand, 1984). Dando *et al.* (1985) have discovered another ecological element pertaining to sulfide-oxidizing symbioses. In a community with the pogonophoran *Siboglinum fiordicum* Webb, which is known to have sulfide-oxidizing symbionts (Southward *et al.,* 1981), three bivalves were found that possessed enzymes in the ctenidia characteristic of the symbiosis: *Lucinoma borealis* Linné, *Myrtea spinifera* (Montagu), and *Thyasira flexuosa* (Montagu). Strikingly, free sulfide in the sandy silt of the environment is lower than 0.5 μM, in contrast with levels of 160 μM to 25 mM reported for Galápagos Rift and *Solemya* environments (Felbeck, 1981, 1983). Sediment-bound sulfide is, however, relatively high, indicating that the organisms are able to draw upon it. Without this source of sulfide there would be insufficient energy to account for known levels of basal metabolism, growth, and reproduction. Dando *et al.* (1985) further suggest that pseudofeces enhance sulfide production, and that thiosulfate derived from iron-bound sedimentary sulfide in the vicinity of the inhalant tube is absorbed for use by the symbiotic bacteria. We would propose the intriguing possibility that bound sulfide is released from ingested particles due to the low pH and anoxic state of the gut. This would then be a third point of possible sulfide absorption in addition to the suprabranchial chamber and pallial gills. We also observe that at our Crofton site *Parvilucina* is limited to sediments with high free sulfide, as determined by the semi-quantitative olfactory test proposed by Dando *et al.* (1985). *Thyasira flexuosa,* which is present there in small numbers, becomes more numerous in contiguous sandy silts with lower free sulfide.

Paedomorphic Characteristics

The loss of the outer demibranchs in the Lucinidae, and the fusion of the subfilamental tissues that house the symbionts, points up a physical requirement of the bi-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valve-bacterial association. In a filter-feeding bivalve the chief criterion for particle collection and selection is a large ciliated surface area, and relative freedom of flow between the infrabranchial and suprabranchial chambers. This requirement is served by the double demibranchs found in most lamellibranchiate bivalves. This large surface area provides for far more respiratory gaseous exchange than these animals would ever require; therefore, the double demibranch gill is primarily a feeding organ. But for symbiotic gill bacteria the primary requirement is an adequate volume of host tissue, albeit arranged in cellular monolayers, to allow access to dissolved sulfide and oxygen both in the blood and mantle water. The lucinid lamellar gill structure is slightly convergent with that of the Solemyidae (Figure 9), but represents a compromise between total commitment to symbiosis and the requirement of a filtering and food-collecting gill for conventional alimentation. STASEK (1963) has observed that the failure to develop a second demibranch is paedomorphic. The hypotrophy of the gut may be a similar manifestation, presenting no dietary embarrassment because of the symbiotic nutritional supplement. However, gut reduction can occur as an independent process, as in *Solemya reidi* and in four other gutless solemyid species discovered by KUZNETSOV & SHILEIKO (1984). Paedomorphosis could also clear up the problem of how the stomach form should be classified. PURCHON (1956, 1957, 1958, 1960) delineates five stomach types and places the lucinid *Lucinoma borealis* and *Thyasira flexuosa,* in the type-IV category. However, ALLEN (1958) clearly shows that in three species of *Diplodonta* (Lucinacea: Ungulinidae) the stomachs belong to Purchon's type V, having the distinctive major typhlosole with two extensions entering a pair of duct caeca. Allen then argues convincingly that the smaller and less complex stomachs of the Thyasiridae and Lucinidae are simplifications of the type-V form found in the Ungulinidae. PURCHON (1978) admits that a number of bivalve stomachs consigned to the rather loose type-IV assemblage may indeed be paedomorphic simplifications of other gastric types. Allen suggests further that gut regression in the Thyasiridae and Lucinidae represents an adaptive trend toward a more macrophagous habit, correlated with their relatively poor environments, a trend similar to that taken by the carnivorous Septibranchia. Although this has a *faute de mieux* plausibility we find nothing in the histochemistry and cytology of the digestive tract to support it, and the food particles found in the *Parvilucina* rarely exceed 40 μm, while the gills reject most particles longer than 75 μm. The proven existence of a nutritive symbiosis in the Lucinidae and the existence of gill bacteria in some Thyasiridae suggest that the simplification of the gut reduction or loss of the outer demibranchs, and gill filament modifications are correlates of symbiosis. *Axinopsida serricata* proves this rule, but we interpret this minute, ubiquitous species as one in which symbiosis has regressed, and r-selected qualities have been emphasized.

Transmission of Symbionts

The method of transmission of the symbiotic bacteria from one generation to the next in *Parvilucina* is not known. In *Solemya reidi* there appears to be an intimate vertical transmission from parent to offspring, with recognizable bacteria developing in the larval test tissues, from which they are released into the mantle cavity (GUSTAFSON, 1985). At this stage of development the larva has unconnected oesophageal, gastric, and rectal rudiments. An oral ciliary feeding organ draws into the oesophagus bacteria and infected fragments of the test tissue, which autolyzes during metamorphosis. When the gut itself degenerates at metamorphosis its population of bacteria is released into the hemocoel, whence they are presumed to be passed to the developing ctenidia. GUSTAFSON (1985) assumes this transmission to be holobiotic, that is, the eggs of *S. reidi* are already infected. In *Parvilucina* the form and size of the recognizable symbiotic bacteria are different from those of *Solemya*. Moreover they are contained in distinct vacuoles, and also are found free in the suprabranchial chamber. ALLEN (1958) notes that the ctenidial pigmented granules in the Thyasiridae are found free between the gill filaments and he describes this as an excretory process. Bacteria may also be released in the same way, becoming available to uninfected intercalary cells in the subfilamental tissue. We do not find uninfected adults, nor any obvious variations in the level of infection. Intimate symbioses tend to produce symbionts that are immune to the defense mechanisms of the hosts, and whose metabolic capabilities have been adjusted to those of the host, through a history of regression of labile enzymes, whose loss could be compensated by the host. Therefore, a transmission mechanism that ensures infection of the young bivalves is almost essential and, although there may be many free-living species of sulfide-oxidizing bacteria, it is unlikely that the transmission mechanism involves a random post-metamorphic infection by such free-living bacteria.

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Figure 8

Transmission electron micrographs of the gills of *Axinopsida serricata* and *Thyasira flexuosa*. A. Subfilamental tissue of *Axinopsida*, composed of storage epithelia (s) with mitochondria. B. Portion of gill filament of *Thyasira* showing central blood sinus (bl) and subfilamental storage cell (s) with distal "bacteriosomes" (bs), vacuoles containing bacteria. C. Storage epithelium (s) with bacteriosome.

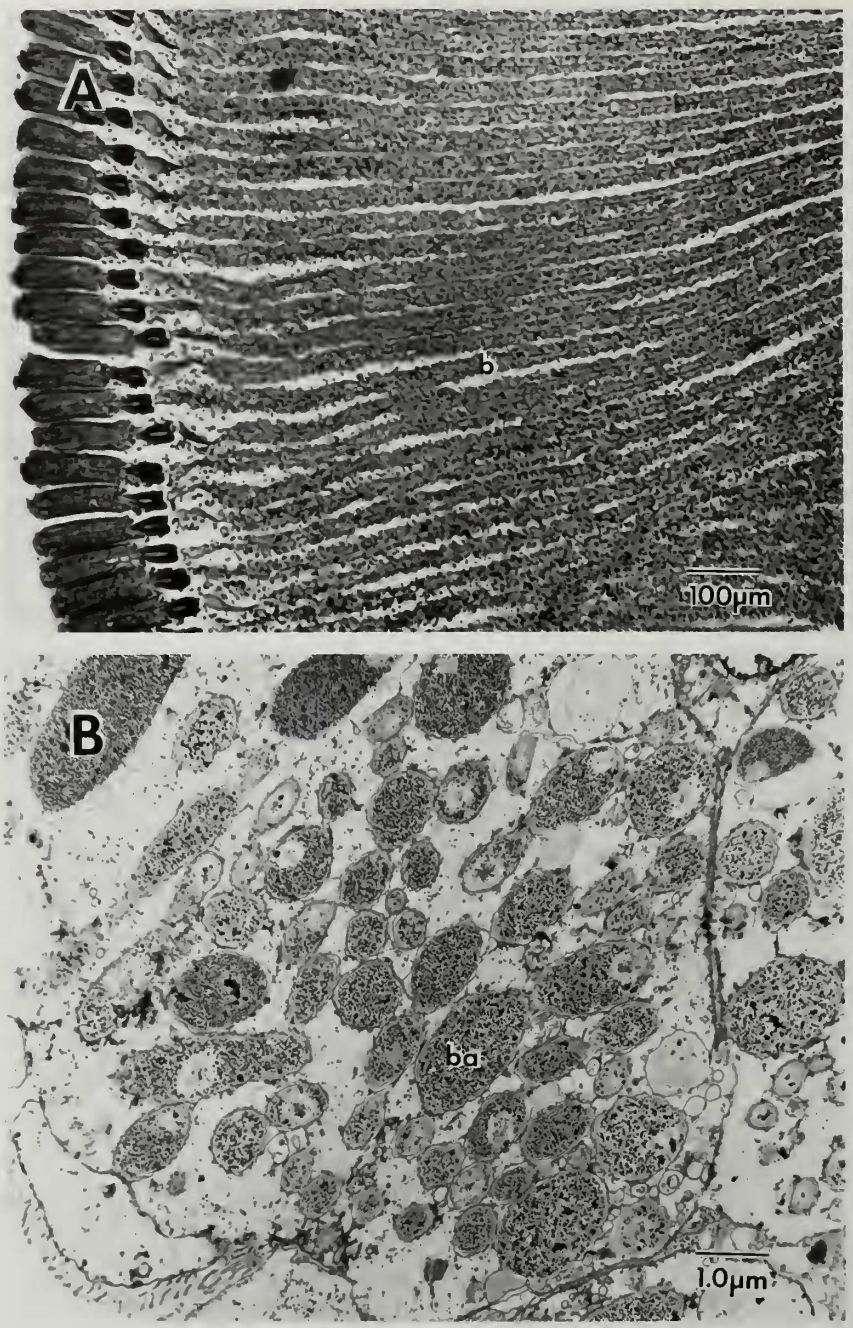

Figure 9

Sections of the gill of *Solemya reidi*. A. Light micrograph of gill filaments; ciliated frontal margins are at left; to right of the chitinous supporting rods the larger portion of the gill consists of bacteriocytes (b). This is the functional analogue of the subfilamental tissues of the Lucinidae and Thyasiridae. B. *Solemya* bacteriocyte. Note the larger bacteria (ba) than those found in *Parvilucina* and *Thyasira*.

The Evolution of the Lucinacea

Firstly we accept the scheme suggested by ALLEN (1958) for the extant forms with the qualification that the evolutionary trends in form and habit have been toward the refinement of symbiosis rather than toward macrophagy. The Ungulinidae represent the primitive form, possessing the most typical eulamellibranch gills and a typical digestive tract morphology, with no gastric simplification, nor expansion of the subfilamental tissue. The Thyasiridae have somewhat expanded the subfilamental tissue, reduced the outer demibranchs, and simplified the gut, and the Lucinidae represent the greatest commitment to symbiosis. The most significant question is where and when was the symbiosis first established? On the face of it, and taking a parsimonious view, the symbiosis should have occurred in an ungulinoid species, with a type-V stomach and double demibranchs, providing a line that then diverged to found the Thyasiridae and the Lucinidae; alternatively, a less likely diphyletic evolution came from two independently symbiotic ancestral species. But this leaves out a fundamental, albeit paraphyletic, character of the Lucinacea, the absence of the inhalant siphon. For a suspension-feeding bivalve inhabiting anoxic silt this would seem to be a reckless omission, reducing the efficiency of filtration and the selectivity of ingestion, increasing the risk of clogging the filtering organs with indigestible matter, and inviting immolation by sulfide. On the other hand, if the bivalve already had the beginnings of a symbiosis with sulfide-oxidizers, even a loose association with bacteria in the mantle cavity and on the gill surfaces, there would be every advantage to the loss of the inhalant siphon, provided that the foot were able to make an adequate ventilation tube for acquiring the oxygen necessary for bacteria and bivalve alike. Adducing these ideas to the evolution of the Lucinacea the ancestral form may already have had the symbiosis, which provided most of the evolutionary drive for the group, with the exception of the modern Ungulinidae; the Ungulinidae would have to be seen as a line that abandoned the symbiosis, which might not have shifted from an ectosymbiotic to an endosymbiotic relationship, before the morphological modifications seen in the other two families had occurred (Figure 10A). One hypothesis would suggest a shallow-burrowing, short-siphoned suspension-feeder as the ancestor, possessing a loose symbiosis that provided the incentive to burrow deeper into anoxic sulfide-generating layers, to lose the inhalant siphon by paedomorphosis, and to develop the foot as an organ for constructing the burrow that would double as a ventilation and feeding route.

The lucinoids are an ancient Palaeozoic group, traceable to the late Early Ordovician, if *Babinka* is a natural relative of the other lucinoids as MCALESTER (1965) has argued (Figure 10B). POJETA (1978) who supports this contention, provides a brief review of the *pros* and *cons*. The radiation of the Lucinacea was a later event, occurring in the late Mesozoic (MCALESTER, 1966). The problem with the stratigraphic evidence, based on shell-form, is that it runs counter to the evolutionary sequence that we are hypothesizing on the basis of functional morphology, and which agrees in general outline with the assessment made by ALLEN (1958). MCALESTER (1966) shows the Lucinidae arising in the Silurian, and the Thyasiridae and Ungulinidae in the Cretaceous (Figure 10B). However, the only shell features that are suggestive of habit are the absence of siphonal muscle scars and the presence of extensive anterior adductor scars, this feature being regarded by Allen as of nutritive significance, because the adductor surface provides a supplementary collecting surface. Coincidentally one of the oldest known bivalve mollusks, *Fordilla troyensis*, found in the upper Lower Cambrian, has similarly extensive adductors (POJETA, 1978). These characters are found in all Lucinacea, so the stratigraphic evidence does not necessarily contradict our hypothesis of habit evolution: the early Lucinidae probably had a morphology like that of the modern Ungulinidae. McAlester's most radical contention is that the lucinoids represent an independent line of bivalve evolution, with the Babinkidae arising from an Early Cambrian monoplacophoran ancestor (MCALESTER, 1966). However, the possession of typical eulamellibranch gills and a type-V stomach by the Ungulinidae undermines both the latter hypothesis and the argument that the functional morphology of the Ungulinidae is the most recent type to appear in the Lucinacea.

Occurrence of Sulfide-Oxidizing Symbiosis in Bivalves

That a symbiosis could provide sufficient momentum for a major evolutionary departure is an example of emergent evolution, a phenomenon discussed elsewhere in the larger context of animal evolution by REID (1985). Malacologists already familiar with the allometric shifts and modifications in the Tridacnidae, whose evolution was spurred by symbiosis with photosynthetic dinoflagellates, will not be surprised at the evolutionary impact of such associations. But with a few minor exceptions the algal-bivalve symbiosis is unique to the Tridacnidae. Has the bacterial-bivalve symbiosis been of more general evolutionary significance? We will commence with the negative evidence.

Although the Tellinacea were generally regarded as specialist deposit-feeders inhabiting coarse substrates (YONGE, 1949), there are many exceptions to this rule (POHLO, 1969). REID & REID (1969) demonstrated three feeding categories in the tellinid genus *Macoma*: coarse sand deposit-feeders, fine silt deposit-feeders, and suspension-feeders. Several species inhabit silts with various sulfide levels. *Macoma carlottensis* is frequently found in the company of *Solemya reidi*, *Parvilucina tenuisculpta*, and *Thyasira flexuosa*, and we double-checked this species from

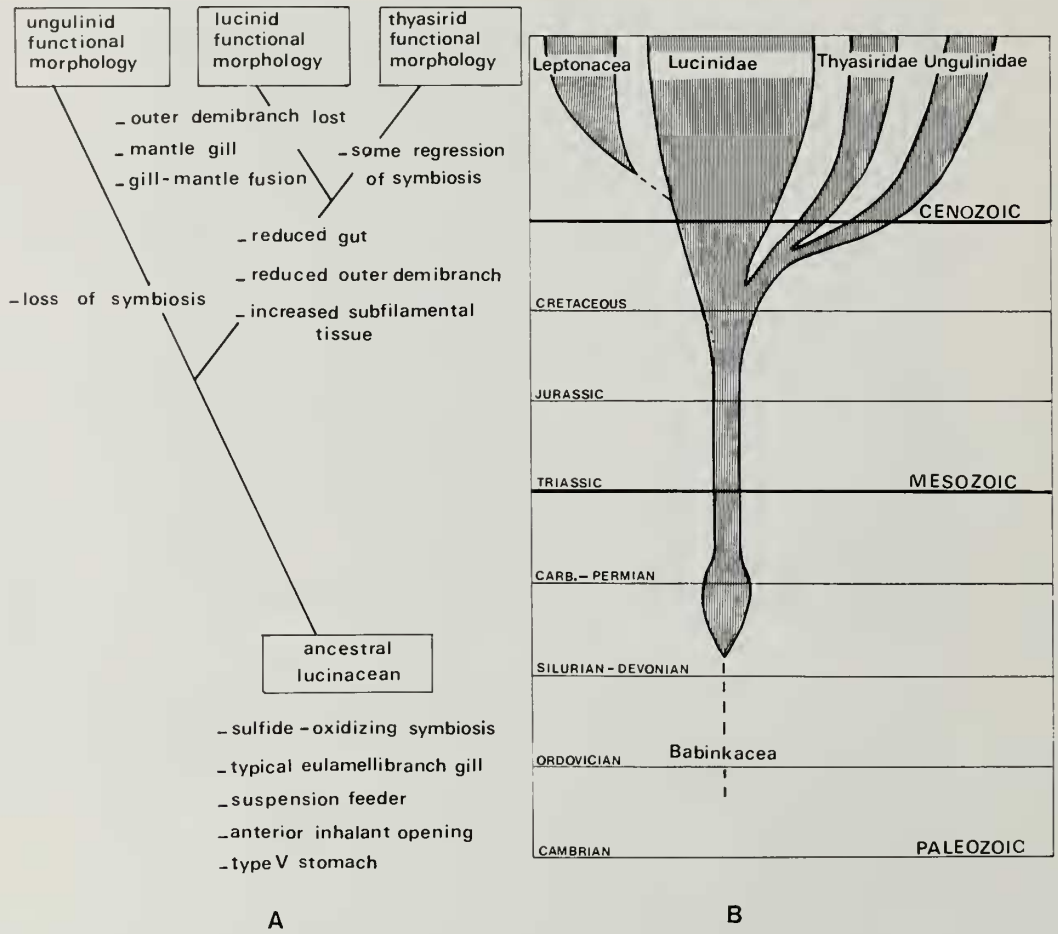

Figure 10

Evolution of the Lucinacea. A. Scheme proposed for the evolution of the Lucinacea. B. Diversification of the Lucinacea (after McAlester, 1966).

our Pipestem Inlet and Crofton sites to confirm that it lacks symbiotic bacteria, like all the other members of the genus that we have examined. *Macoma nasuta* was included in the enzymological survey by Felbeck *et al.* (1983) and found negative. Other common inhabitants of anoxic benthic muds, such as the cockle *Fulvia hungerfordi* (Reid & Shin, 1985) and the venerid *Compsomyax subdiaphana,* also lack the ctenidial bacteria. This symbiosis is neither casual nor random. Its original establishment seems to have depended on access to numerous potential symbionts, together with a regular supply of oxygen and sulfide. In burrowing, benthic bivalves the inhalant siphon would likely impede sulfide uptake from the burrow. On the other hand, an anterior inhalant current, or simply the pedal aperture, provides a potential route for sulfide absorption if the normal tendency to "clam-up" in the presence of this molecule is inhibited.

In *Calyptogena* (Vesicomyidae) the siphons are short and the pedal aperture large (Figure 11). Bernard (1974), Boss & Turner (1980), and Morton (1985) note the presence in *Calyptogena* spp. of expanded subfilamental gill tissue, suggesting that the genus arose from a symbiotic ancestor. Boss & Turner (1980) did not find food grooves in *Calyptogena magnifica* and consider this a correlate of the symbiotic habit. Morton (1985) identifies a small food groove at the margin of the inner demibranch of *C. magnifica* and argues that this feature, together with a gut and digestive diverticula of "normal" proportions, indicates that the digestion of free-living bacteria is an important part of this bivalve's alimentation, and that its dependence on symbiotic bacteria has perhaps been exaggerated. Nevertheless, Fiala-Medioni (1984) has identified numerous gill bacteria in this species. Arp *et al.* (1984) have shown that the vascularized foot of *C. magnifica* is protruded down between the lumps of debris of the hydrothermal vent regions to reach interstitial water with a high sulfide content. At the same time it can take in oxygen through the inhalant siphon. In the vent mytilid

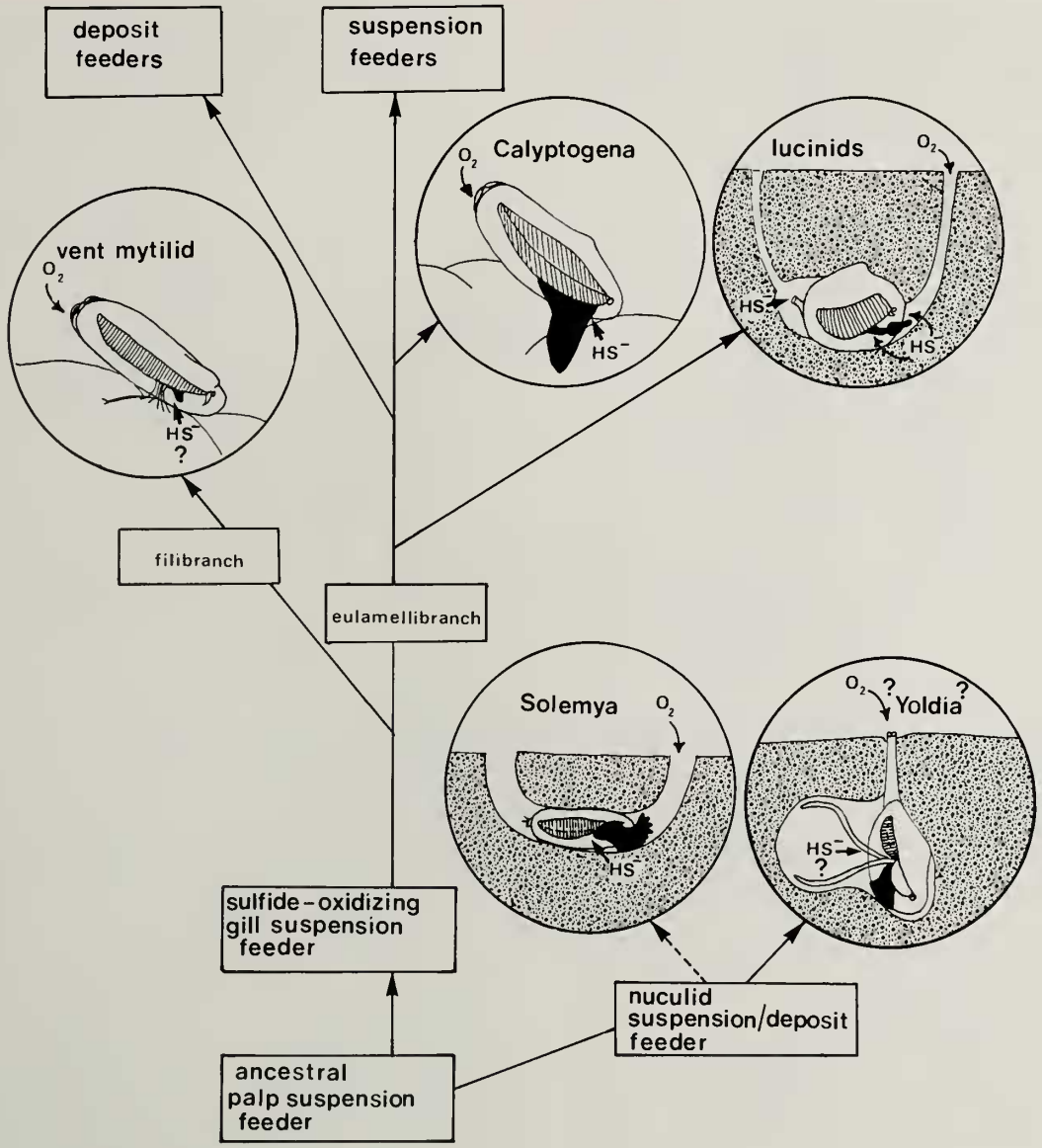

Figure 11

Distribution of sulfide-oxidizing symbiosis in relation to bivalve evolution. Gills are indicated with vertical hatching, feet in solid black; palps are indicated to the anterior (right) of the gills. The sketch of the lucinid is based on ALLEN (1958); that of *Calyptogena* is based on ARP *et al.* (1984) and MORTON (1985). The *Yoldia* drawing is after BENDER & DAVIS (1984). The inclusion of *Yoldia* in this scheme is debatable, because it has not been established that the few bacteria in the gills are sulfide-oxidizing.

Bathymodiolus thermophilus (Kenk & Wilson, 1985) there may be a similar route of sulfide acquisition. LE PENNEC & HILY (1984) report that the gills of this organism have a bacterial epiflora as well as intracellular symbionts. The gill filaments are broad and fused ventrally to form septa, and distinct food grooves are present. KENK & WILSON (1985) note that the gut is simplified. We observe, as do KENK & WILSON (1985), that the mantle edges of this species are fused between the pedal aperture and the inhalant siphons. This may be correlated with the partitioning of sulfide and oxygen. It is obvious that in a crowded community where the primary producers are sulfide-oxidizing bacteria, and where one or more of the metazoa have entered mutualistic symbioses, there is great potential for this type of association to become established in other members of the community, through interphyletic

transmission of symbionts. Interestingly, HICKMAN (1984) identifies a distinct *Thyasira-Lucinoma-Solemya* community from Cenozoic deep-water deposits. This could also be characterized as a sulfide-oxidizing community, similar to what we find at the present time.

The Significance of the Anterior Inhalant Current

In the Lucinacea, the anterior inhalant opening and absence of the inhalant siphon are more likely paedomorphic than primitive. Members of another bivalve assemblage, the Leptonacea, which MCALESTER (1966) presents as a possible offshoot of the Lucinacea (Figure 10B), also lack the inhalant siphon and have an anterior inhalant opening; in some a short, anterior inhalant siphon is present. Members of the Leptonacea, a group of debatable lineage, comprising small, ephemeral bivalves on the whole, are generally regarded as paedomorphs subject to r-selection. This condition would also be receptive to sulfide-oxidizing bacteria, and once a symbiosis were established an increase in size and prolongation of the lifespan could follow. Although one species of the leptonacean genus *Kellia* is known to lack the usual symbiotic enzymes (FELBECK *et al.,* 1981), members of this group from high sulfide silts deserve further examination.

In other bivalves the juvenile habit involves an anterior feeding current created by the pedal ciliation. Indeed, OCKELMANN & MUUS (1978) point out that there are no known exceptions, observing that this is a necessary means of separating inhalant from exhalant flow in minute bivalves. Details of this ciliation are available for *Macoma balthica* (CADDY, 1969), *Mytilus edulis* (BAYNE, 1971), and *Abra alba* (AABEL, 1983). In *Panope abrupta* juveniles, pedal ciliation and muscular activity direct a stream of food particles through the pedal aperture on to the labial palp surfaces (KING, 1985, and personal communication). In mature *Mysella bidentata* the inhalant current has been described by OCKELMANN & MUUS (1978) and pedal detritus-feeding in this species is discussed by O'FOIGHIL (1981). McMahon (personal communication) has discovered that adult *Corbicula fluminea* are pedal detritus-feeders. AABEL (1983) observes that juvenile *Abra alba* ingest deposit material anterior to the foot, and that they continue to feed in anoxic deposits. If this is a common habit in juvenile Tellinacea it is all the more surprising that the most opportunistic genus *Macoma* has not taken up with sulfide-oxidizing symbionts, especially *Macoma carlottensis,* which is so frequently found in the company of *Solemya reidi, Parvilucina tenuisculpta,* and *Thyasira flexuosa.* We conclude that the specialized feeding behavior and functional morphology of this and other species is an obstacle to the sulfide uptake necessary for symbiosis, despite the potential susceptibility of the juveniles.

In the Protobranchia, which include the Solemyidae, the anterior inhalant opening and the absence of an inhalant siphon are regarded as a primitive condition. LILJEDAHL (1984) has proposed the Silurian protobranch *Janeia silurica* as an intermediate between nuculoids and solemyoids, possessing smaller gills than modern symbiotic *Solemya* species. Somewhere along this evolutionary line the symbiotic association was formed, allowing the enlargement of the aspidobranchiate gills, regression of the labial palps and gut, and modifications in burrowing and ventilation behavior, features that the Lucinacea have partially paralleled. Indeed, gill enlargement and palp reduction are evolutionary processes that have been followed in all bivalves, and the question that we wish to pose here is whether or not sulfide-oxidizing symbiosis might have had a much more central role in bivalve evolution than has hitherto been suspected. To begin to answer this it is first necessary to consider the more general question of the habit of the most primitive protobranch bivalves.

Feeding Habits of the Nuculidae

The detritus-feeding habit of the recent Nuculidae, together with their shell dentition, are taken to represent the founding condition of the Bivalvia by YONGE (1939). However, STASEK (1965) argues from a study of *Acila castrensis* that the nuculids were primitively suspension-feeders, their gills being functional food-collecting organs, and that the detritus-collecting palp proboscides are a secondary development of the terminal posterior ciliated ridges of the food-sorting palp lamellae. On the basis of previously unpublished observations of *Nucula sulcata* by the senior author we agree with Stasek. Specimens of *N. sulcata* whose palp proboscides had been surgically removed in the manner employed by STASEK (1961), and which had been mounted in a suspension of *Aquadag,* effectively filtered and ingested graphite particles. The palp lamellae are the primary feeding organs; their complex, ciliated inner surfaces collect and sort suspended particles from the anterior inhalant current (Figure 12). The gills, being small, are less significant collecting organs. The detritus-collecting palp proboscides are, as Stasek suggests, an evolutionary after-thought. As we have already pointed out, feeding from an anterior inhalant current is a universal characteristic of post-larval bivalves and, in the cases noted above, the relatively large labial palps are the primary collecting and sorting organs, prior to the growth of the gills and their establishment as the most important filtration site. An anterior inhalant stream might be regarded as a necessary consequence of small size, and the primary role of the labial palps interpreted as a necessary juvenile specialization. But it must be remembered that these are also the primitive arrangements and are in all likelihood a case of ontogeny repeating phylogeny. We would argue that the primitive protobranchs received a mixture of suspended and deposit particles that were collected and sorted by the palps and that the Nuculidae became specialized detritus-feeders with the development of the palp proboscides. We find no bacteria in the gills or palps of the nuculid *Acila castrensis* and assume that this is representative of the nuculid condition.

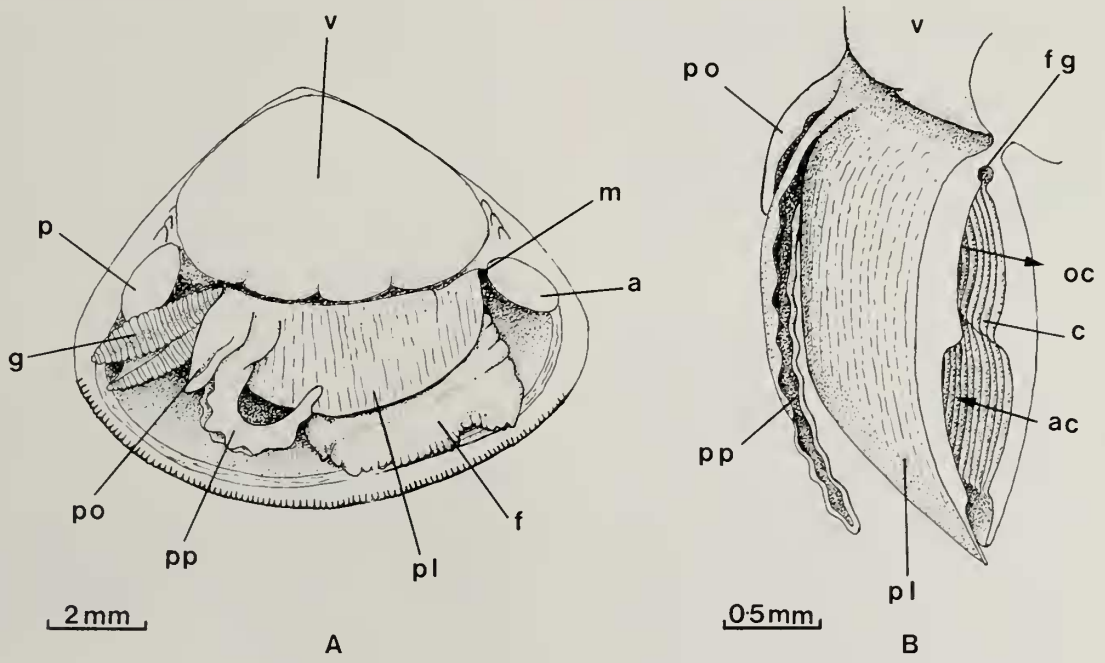

Figure 12

The feeding apparatus of *Nucula sulcata*. A. Right lateral view of animal with mantle removed. B. Solid section of the labial palp complex looking toward the posterior. The aboral current (ac) contributes to the general anterior inhalant current. The oral current (oc) is an interrupted flow due to the appression of the high ridges of the dorsal regions of the interior of the palp lamellae. a = anterior adductor muscle; ac = aboral current; c = ciliated ridge of the palp lamellar interior; f = foot; fg = food groove; g = gill; m = mouth; oc = oral current; p = posterior adductor muscle; pl = palp lamella; pp = palp proboscis; po = palp pouch; v = visceral mass. Details of the sorting ciliation of the palps are similar to those of *Acila castrensis* (STASEK, 1961).

The Protobranchia and Early Bivalve Evolution

The nuculanid Protobranchia, and *Yoldia* in particular, have continued in the trend established by the nuculids, but with more effective ctenidial food collecting (STASEK, 1965). Stasek has also elaborated an interesting ctenidial function noted by DREW (1899) and YONGE (1939). The gills can act as a muscular pump, drawing water into the infrabranchial chamber, and also, via the exhalant siphon, into the suprabranchial chamber. Moreover, although the Nuculanidae have an inhalant siphon, one species at least, *Yoldia limatula,* can create a subsurface chamber with the proboscides, as BENDER & DAVIS (1984) have demonstrated (Figure 11). All of these features suggest the possibility of a sulfide-oxidizing symbiosis in this genus. However, although we have identified bacterium-like bodies in some cells of the ctenidia of *Yoldia scissurata,* we do not know if these are capable of sulfide-oxidation, and even if this proves to be the case, the symbiosis has either regressed or failed to progress to the point where there are many well-defined bacteriocytes capable of making a significant contribution to the nutrition of the species.

Generalizing on the subject of early lamellibranchiate bivalve evolution PURCHON (1978) writes, "The emergence of this filter-feeding model was a mega-evolutionary change: The stuff of which subclasses are made!" As to what this new model emerged from, there is no positive evidence for the traditional view that the founding habit was infaunal detritus-feeding. We prefer the suggestion of a palp suspension-feeder, quite possibly an epifaunal bivalve on a hard surface as inferred by STASEK (1972, and personal communication): significantly most other bivalved and quasi-bivalved invertebrates, Ostracoda and some Branchiopoda (Crustacea) and Brachiopoda, are epifaunal or planktonic suspension-feeders. Exceptions are the Juliidae, bivalved gastropods that are specialized suctorial browsers on macroalgae. The functional correlates of the bivalve form are protection of the enlarged, vulnerable food-collecting organs, and energetic efficiency in generating feeding currents. Invertebrate detritus-feeders are more commonly wormlike burrowers.

If the bivalves arose monophyletically from an aspidobranchiate, suspension-feeding ancestor whose primary food-collecting organs were the labial palps, how did the switch of primary feeding function to the gills occur? A sudden allometric shift might have been responsible. A sulfide-oxidizing symbiosis might also have triggered ctenidial expansion, as in the Solemyidae, with the new po-

tential for more efficient suspension-feeding being realized before the hypotrophy of the gut became irreversible. The vascularization of the gills would make them a likely repository for blood-borne symbionts. Possibly these initially provided an exogenous epigenetic growth stimulus for the gills, which was finally genetically assimilated. The sieving mechanism of the ctenidial filter, if large enough, would certainly be more effective than the palp surfaces, providing the new model extolled by Purchon.

CONCLUSIONS

The Bivalvia are not necessarily the only class of Mollusca that have experimented with sulfide-oxidizing symbiosis. The scaphopod *Dentalium rectius* is commonly found in association with *Parvilucina tenuisculpta* and *Thyasira flexuosa,* and like other scaphopods has the potential for a regulated influx of sulfide and oxygen. Moreover, concentrations of "symbiotic" bacteria have been found in the polar region of scaphopod eggs (GEILENKIRCHEN *et al.,* 1971; DUFRÈSNE-DUBE *et al.,* 1983). Bacterial colonization of the gills of a new species of archaeogastropod limpet from the Juan de Fuca hydrothermal vents has been noted by DE BURGH & SINGLA (1984). Their study indicates two possible routes of entry for an incipient symbiont: the gill epithelia endocytose and digest the bacteria, and also the hypotrophic gut contains similar bacteria. De Burgh and Singla admit the possibility that if some bacteria were resistant to digestion by the host a mutualistic symbiosis could result, although they are disinclined to conclude that the subject of their study is on the road to endosymbiosis. Nevertheless, this route to a symbiotic destination seems to have been taken by *Solemya reidi.* WILKINSON (1984) has argued that, because according to immunological analysis the bacterial symbionts in sponges are closely related, symbiosis must have been a unique Precambrian event. This does not preclude transspecific infection within the phylum, nor even interphyletic transmission, as may be the case in the dinoflagellate symbionts of reef corals and giant clams (FITT & TRENCH, 1981) and in the hydrothermal vent communities (STAHL *et al.,* 1984). The latter authors also find that the symbionts of the intertidal *Solemya velum,* though morphologically distinct, are related to the hydrothermal vent community symbionts in terms of ribosomal RNA sequence similarities.

In conclusion, one, or a small number of free-living species of sulfide-oxidizing bacteria, seems to have had the potential for endosymbiosis with sedentary marine invertebrates living at the interface between an aerobic environment and a sulfide-generating anoxic environment. Once established, the potential for interphyletic transmission was realized. Amongst the bivalves the primitive anterior inhalant feeding current, or its paedomorphic retention, together with the absence of an inhalant siphon, seem to have enhanced susceptibility to the symbiosis. Paedomorphic gill and gut structures were other correlates, along with the development of subfilamental tissue to accommodate bacteria and the ability to partition the supply of oxygen and sulfide either physically or temporally. As far as the bacteria are concerned the bivalve is a dependable interface between the anaerobic and aerobic environments, the obligatory sources of their metabolic needs, releasing them from vacillations and other vicissitudes of the free-living condition. These symbioses have provided the major evolutionary drive for the emergence of the Lucinacea and the Solemyidae, and they may have participated in other areas of molluscan evolution.

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